THE MICRO-COLORIMETER IN THE INDICATOR METHOD OF HYDROGEN ION DETERMINATION

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In a recent paper[1] attention was drawn to the use of the colorimeter in determining accurately by the indicator method the hydrogen ion concentration of pigmented biological fluids. In the method there discussed there was employed the well-known principle of compensating for the color of the test solution by introducing the test solution also as a shield solution. It was pointed out that in order to avoid the optical difficulties of the usual tintometers or comparators, while retaining all the advantages of the colorimeter, glass cells fitting one within another are arranged as cup and shield respectively. Properly made these cells are expensive, and the method requires about 15–20 cc. of the liquid for convenient determinations by the colorimeter.

Recently I have had occasion to test the hydrogen ion concentration of some fluids obtainable only in small quantity, and while examining the possibilities of adapting the micro-colorimeter for this work it became clear that the Dubosq type of this instrument lends itself admirably to the colorimetric method in general, and to small quantities of fluid in particular. Moreover, as the method is now modified the necessity for special cells is eliminated. Instead of employing two special cells in connection with each plunger of the colorimeter the principle of the new procedure lies in the use of the plunger tube and of a colorimeter cup as cells on each side of the system. When the quantity of test material available is not limited it is customary in our work to employ 5-cc. quantities, and 5-cc. quantities of the standards, the solutions being prepared in small serological test-tubes to each of which is added 3 drops of indicator. With

[1] Duggar, B. M., and Dodge, C. W. The use of the colorimeter in the indicator method of H ion determination with biological fluids. Ann. Mo. Bot. Gard. **6**: 61–70. *f. 1.* 1919.

the micro-colorimeter a quantity as small as 1 cc. of sample may serve both for test solution and for the shield. It is preferable, however, to have not less than 2 cc. for most careful work.

The standardization of the apparatus for this work is extremely simple. It is merely necessary to know the volume of the plunger tube and its length so that in the determinations it will be possible to place a given volume of solution in the plunger cylinder, and knowing the depth which this will occupy, the instrument may be set so that a similar depth will be examined in the colorimeter cup. This is important, since in one case, as described later, the pigmented sample is placed in the plunger tube and in the other case in the colorimeter cup. Therefore equal depths of solutions will be examined in both cells of the systems. In the instrument at our disposal the plunger tube is 33 mm. in length and the volume 1.25 cc. Since the tube is cylindrical the volume is proportional to length, so that if .625 cc. of solution is added the column has a depth of 16.5 mm. The depth need not be so great as this, and 0.5 cc. of liquid is sufficient. If the quantity of the solution employed is reduced beyond this point, it is necessary to increase relatively the amount of indicator added. Where the total quantity of the test solution is 2 cc., 1 cc. being employed for the sample and 1 cc. for the shield, we find it desirable to use 1 drop of indicator for the 1-cc. sample. If this proves too highly colored the indicator may, of course, be diluted one-half.

I find it desirable to arrange the samples, standard, and shields as follows:

> In the left plunger tube place the measured quantity of plain water as shield, and in the colorimeter cup of that side place a quantity of the sample or test solution plus indicator which shall give any depth greater than that of the liquid column in the plunger tube. In the right plunger tube place the measured quantity of the sample as shield, and in the right colorimeter cup the standard solution plus indicator.

With this arrangement it is desirable to make up a few standards covering the range of probability, and then in making the determination it is only necessary to change the solution in the right colorimeter cup until an exact match is obtained. There

is one slight optical defect due to the fact that the surfaces of the liquids in the plunger tubes are not plane surfaces, but this is of no practical consequence in the actual determination, especially where a strong and standard source of light is employed. It is recommended that any of the so-called daylight bulbs be employed in this work. Using the method mentioned no difficulty whatever has been experienced in determining rapidly and effectively the hydrogen ion concentration of such dark liquids as oxidized potato juice, carrot decoction, and decoctions of plants containing considerable chlorophyll.

It is perhaps unnecessary to add that the technique suggested is equally applicable where the large types of colorimeters are employed, such as the Dubosq of standard size, or the Kober (if plunger tubes are detachable), but somewhat larger quantities of solutions will be required.

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